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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM **6(A)(2) DATA**

SUBJECT: **GLUTARALDEHYDE. ID# 403901**. Evaluation of a Combined Chronic Toxicity/Carcinogenicity Drinking Water Study in Rat.

Tox. Chem. No.: 468
PC Code No.: 043901
DP Barcode No.: D202936
Submission No.: S464876

FROM: Linnea J. Hansen, Ph.D. 12/7/95
Section IV, Toxicology Branch I
Health Effects Division (7509C)

TO: Virginia Dietrich, Manager, PM Team 51
Ron Kendall, Reviewer, PM Team 51
Special Review and Reregistration Division (7508W)

THROUGH: John Doherty, Ph.D., D.A.B.T., Acting Section Head 12/8/95
Section IV, Toxicology Branch I
Health Effects Division (7509C) 3/15/96

Karl Baetcke, Ph.D., Branch Chief
Toxicology Branch I
Health Effects Division (7509C)

I. CONCLUSIONS

TB-I has reviewed the chronic toxicity/carcinogenicity drinking water study in rats on glutaraldehyde and the results are summarized below:

EXECUTIVE SUMMARY: In a chronic toxicity/carcinogenicity study (MRID 43191101), 80 Fischer 344 rats/sex/dose group were administered glutaraldehyde (technical, 50% a.i., aqueous solution) in their drinking water at concentrations of 0, 50, 250 or 1000 ppm glutaraldehyde for 104 weeks. Doses correlated to an average daily glutaraldehyde intake of 12.3, 49.2 or 126.1 mg/kg/day in males and 15.0, 60.8 or 154.6 mg/kg/day in females. An

additional 10 animals/dose/sex were included in each of two satellite groups sacrificed at 52 and 78 weeks.

At 50 ppm, marginal decreases in water consumption (-2 to -12%) were observed which were probably related to unpalatability of glutaraldehyde. At 250 ppm, decreased body weight gain in males (-14%, week 103) and slightly decreased food consumption (both sexes, -2 to -8%) were observed. Urine volume was decreased and osmolality increased, probably secondary to reduced water intake. Incidence of renal tubular pigmentation was increased in females (probably secondary to large granular lymphocyte leukemia). At 1000 ppm, the incidence of lesions consistent with gastric irritation was slightly increased in both sexes and renal tubular pigmentation and bone marrow hyperplasia in males were increased. In females, body weight gain was decreased (-13%) and absolute and/or relative kidney weights were increased (relative kidney weights only increased slightly in males at weeks 52 and 78). **The LOEL for systemic toxicity is 250 ppm (49.2 mg/kg/day), based on slightly decreased body weight/weight gain (males) and food consumption (males and females). The NOEL is 50 ppm (12.3 mg/kg/day).**

Statistically significant increases in the incidence of large granular lymphocyte leukemia were observed in all treated female groups (41%, 41% and 53% vs 24%, controls). Slight, non-significant increases in incidence of mesothelioma in treated males (1%, 3% and 6% vs. 1%, controls) and uterine adenocarcinoma in females (0%, 4.4% and 2% vs. 0%, controls; about half of low and mid dose animals examined) were also observed. The HED RfD Peer Review Committee will determine whether the increase in tumors in females requires an assessment by the Cancer Peer Review Committee.

This study is classified as **Acceptable** and satisfies the guideline requirements for a chronic toxicity/carcinogenicity study in rat (83-5, or 83-1a and 83-2a).

Action to be taken in response to 6(a)(2) adverse effects: The study identified a possible increase in the incidence of tumors (see above) in female rats. The study will be reviewed by the Agency RfD/Peer Review Committee to determine whether the increase in tumors observed in females will require assessment by the Cancer Peer Review Committee.

II. ACTION REQUESTED

TB-I received for review from Union Carbide Chemicals and Plastics Company a combined chronic toxicity/carcinogenicity study in rats on Ucarcide 250 Antimicrobial (glutaraldehyde) in support of reregistration of this chemical. The study was submitted as 6(a)(2) data because a statistically significant increase in the incidence of large granular lymphocytic (LGL) leukemia was observed in treated female rats. TB-I notes that a draft report for this study was submitted as 6(a)(2) data prior to submission of the final report (MRID 42047601; see memorandum of 11-7-91 from L. Hansen and M. Copley to J. Lee and Velma Noble).

EPA Reviewer: Linnea J. Hansen, Date 12/6/95
Review Section IV, Toxicology Branch I (7509C)
EPA Secondary Reviewer: John Doherty, Acting Section Head, Date 12/8/95
Review Section IV, Toxicology Branch I (7509C)

DATA EVALUATION REPORT

STUDY TYPE: Chronic toxicity/carcinogenicity in rodent (83-1a and-2a)

TOX. CHEM. NO: 468

P.C.CODE.: 043901

MRID NO.: 43191101

TEST MATERIAL: Glutaraldehyde, technical

SYNONYMS: 1,5-pentanedial; glutaric dialdehyde

STUDY NUMBER: 91U0012

SPONSOR: Union Carbide Chemicals and Plastics Company, Inc., Danbury, CT

TESTING FACILITY: Bushy Run Research Center, Export, PA

TITLE OF REPORT: Glutaraldehyde: Combined Chronic Toxicity/Oncogenicity Study in the Drinking Water of Rats

AUTHOR: S.J. Hermansky and K.A. Loughran

REPORT ISSUED: March 18, 1994

EXECUTIVE SUMMARY: In a chronic toxicity/carcinogenicity study (MRID 43191101), 80 Fischer 344 rats/sex/dose group were administered glutaraldehyde (technical, 50% a.i., aqueous solution) in their drinking water at concentrations of 0, 50, 250 or 1000 ppm glutaraldehyde for 104 weeks. Doses correlated to an average daily glutaraldehyde intake of 12.3, 49.2 or 126.1 mg/kg/day in males and 15.0, 60.8 or 154.6 mg/kg/day in females. An additional 10 animals/dose/sex were included in each of two satellite groups sacrificed at 52 and 78 weeks.

At 50 ppm, marginal decreases in water consumption (-2 to -12%) were observed which were probably related to unpalatability of glutaraldehyde. At 250 ppm, decreased body weight gain in males (-14%, week 103) and slightly decreased food consumption (both sexes, -2 to -8%) were observed. Urine volume was decreased and osmolality increased, probably

secondary to reduced water intake. Incidence of renal tubular pigmentation was increased in females (probably secondary to large granular lymphocyte leukemia). At 1000 ppm, the incidence of lesions consistent with gastric irritation was slightly increased in both sexes and renal tubular pigmentation and bone marrow hyperplasia in males were increased. In females, body weight gain was decreased (-13%) and absolute and/or relative kidney weights were increased (relative kidney weights only increased slightly in males at weeks 52 and 78). **The LOEL for systemic toxicity is 250 ppm (49.2 mg/kg/day), based on slightly decreased body weight/weight gain (males) and food consumption (males and females). The NOEL is 50 ppm (12.3 mg/kg/day).**

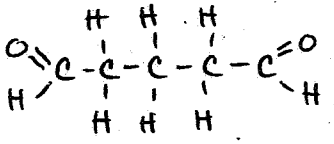
Statistically significant increases in the incidence of large granular lymphocyte leukemia were observed in all treated female groups (41%, 41% and 53% vs 24%, controls). Slight, non-significant increases in incidence of mesothelioma in treated males (1%, 3% and 6% vs. 1%, controls) and uterine adenocarcinoma in females (0%, 4.4% and 2% vs. 0%, controls; about half of low and mid dose animals examined) were also observed. The HED RfD Peer Review Committee will determine whether the increase in tumors in females requires an assessment by the Cancer Peer Review Committee.

This study is classified as **Acceptable** and satisfies the guideline requirements for a chronic toxicity/carcinogenicity study in rat (83-5).

Special Review Criteria (40 CFR 154.7) None

A. MATERIALS:

1. Test Material: Ucarcide® 250 Antimicrobial (Glutaraldehyde, technical)
   Description: colorless liquid
   Lot/Batch #: BRRC Nos. 51-284A, B and C and BRRC 51-528A and B, 52-416A and B and 52-700A and B
   Purity: 50-51% a.i., aqueous, reported by sponsor (see Appendix I of study report. Report indicated approximately 99% purity of a.i. in solution).
   Stability of compound: Stable when stored refrigerated.
   Vehicle: none
   CAS #: 111-30-8
   Structure:



2. Test animals: Species: rat
   Strain: Fischer 344

Age and weight at study initiation: 6 weeks old at study start; Males weighed from 131 to 182 g and females between 98 and 124 g
Source: Charles River Laboratories, Inc., Kingston, NY
Housing: individual stainless-steel wire mesh cages (location rotated every 2 weeks)
Environmental conditions: Temperature 66 to 77°C. Humidity 40% to 70%. Light cycle: 12 hr on/12 hr off. Air changes: not indicated
Acclimation period: 3 weeks
Diet: Purina Certified Rodent Chow #5002

B. STUDY DESIGN:

1. Animal assignment: Animals were randomly assigned to the following test groups:

TABLE 1: ANIMAL ASSIGNMENT

Test Group	Dose Level		Main Study		52 wk sac		78 wk sac	
	ppm	mg/kg/day[1]	♂	♀	♂	♀	♂	♀
Control	0	0	80	80	10	10	10	10
Low Dose	50	4♂, 6♀	80	80	10	10	10	10
Mid Dose	250	17♂, 25♀	80	80	10	10	10	10
High Dose	1000	64♂, 86♀	80	80	10	10	10	10

1 Average daily compound intake calculated by study authors based on water intake and body weight data

Dose selection rationale: Doses were selected based on the results of a 90-day rat drinking water study (BRRC Report no. 48-107) in which Fischer 344 rats were administered glutaraldehyde at 0, 50, 250 or 1000 ppm. At 1000 ppm and to a marginal extent at 250 ppm, decreased water consumption (probably related to taste aversion and sensory irritation), decreased body weight, decreased food consumption, and decreased urine volume/increased specific gravity were observed. Absolute and relative kidney weights were increased at 250 and 1000 ppm but no corresponding gross or microscopic pathology was observed. Animals did not show persisent effects after a 4-week recovery period. The study authors considered 1000 ppm to be a minimally toxic dose and 250 ppm to represent a marginal physiological response.

2. Test material dosing preparation and analysis: Test material was administered in drinking (tap) water. The 50 ppm group was changed to Milli-Q® filtered water and later to Culligan® deionized water after about 6 months due to instability of low concentrations of glutaraldehyde in tap water (attributed to variations in pH). For all dose levels a concentrated premix of 10,000 ppm was prepared, then diluted. Solutions were prepared weekly in large carboys for all dose levels. Dosing solutions were analyzed for stability prior to initiation of the study at 50 and 1000 ppm as stored in Nalgene® carboys and cage water bottles. Homogeneity was determined prior to the study at all dose levels. All analyses were conducted using gas chromatography.

Concentration was measured weekly for the first 4 weeks and every 4 weeks thereafter. At least two samples per carboy were analyzed and the mean values for each carboy were reported individually. Samples drawn for analysis were stored under refrigeration until analysis.

Results - Concentration: Occasional variability was observed in dosing solution concentrations obtained from different carboys at 50 and 250 ppm. At 50 ppm, variation exceeding 10% of target concentration was observed for weeks 17-20, 22, 27, 31, 35, 36, 85 and 94. Most of these variations were decreases relative to target, ranging from -36 to -12% less than target concentration. On a few occasions, increases ranging from +14% to +24% above target were observed. At 250 ppm, variation exceeding 10% of target concentration was observed for weeks 17, 23, 31, 35, 36 and 41. Most of these variations were increases, ranging from 0.4% to as high as 60.8%, on one occasion, above the target except for a 14% decrease in one carboy on week 17. The analytical measurements of the 1000 ppm solutions were within 10% of target concentration. TB-I agreed with the study author that although several wide discrepancies were noted, these variations would not significantly affect the integrity of the study or interpretation of the results.

Homogeneity: The test material was determined to be homogeneously distributed in water for all three concentrations tested. Mean $\pm$ S.D. values at 50, 250 and 1000 ppm were 96.4 ± 0.4, 98.1 ± 0.1 and 97.5 ± 0.5, respectively. There were no outlier measurements.

Stability: Glutaraldehyde was determined to be stable for at least 21 days when stored at room temperature in either the cage water bottles or the storage carboys. All values were within 5% of Day 0 concentration, usually better and this variation appeared to be within experimental error and not due to instability.

3. Animals received food and water ad libitum.

4. Statistics - Data for quantitative continuous variables were analyzed using Levene's test for equality of variances, ANOVA and t-tests when the F value of ANOVA was significant. A pooled t-test was used for pairwise comparisons when Levene's test indicated similar variances and ANOVA was significant. When variances were heterogeneous, ANOVA for unequal variances with a separate variance t-test for pairwise comparisons when necessary. Nonparametric data were analyzed using the Kruskal-Wallis test and Mann-Whitney U test when appropriate. Incidence data were compared using the Fisher's Exact test. A dose-response trend for incidence of LGL leukemia severity grade in the spleen was performed to determine association with increasing dose of glutaraldehyde. The analysis of Farrar and Crump, 1988 and 1990, was used for tumor incidence data to determine presence of any carcinogenic effect

with respect to tumor type, organ site and sex. Statistical significance was assigned where $p < 0.05$ (two-tailed).

5. Signed and dated quality assurance and GLP statements were present.

C. METHODS AND RESULTS:

1. Observations

Animals were inspected twice daily for signs of mortality; a cageside exam for overt clinical signs was performed once daily. A complete physical examination was performed weekly.

Results - Selected clinical signs are shown below in Table 2:

TABLE 2: SELECTED CLINICAL SIGNS[1]

Clinical sign/sex	0 ppm	50 ppm	250 ppm	1000 ppm
MALES:				
Urine stains	14 (293-735)	21 (229-726)	14 (468-738)	26 (41-734)
Emaciation	9 (469-740)	9 (230-733)	10 (510-738)	11 (538-738)
Labored respiration	14 (469-740)	13 (229-732)	10 (636-721)	12 (584-734)
Body pallor	9 (475-732)	11 (423-699)	12 (608-739)	11 (458-732)
Yellow cutis	3 (629-739)	2 (545-685)	3 (721-738)	5 (505-733)
FEMALES:				
Urine stains	22 (71-743)	29 (8-727)	21 (364-733)	38 (22-743)
Emaciation	7 (540-743)	22 (424-722)	13 (342-734)	20 (476-743)
Labored respiration	9 (455-726)	18 (447-712)	17 (358-738)	14 (483-727)
Body pallor	11 (449-732)	17 (421-727)	19 (337-727)	16 (463-743)
Yellow cutis	3 (629-739)	6 (242-708)	5 (568-738)	7 (625-739)

1 Data extracted from Tables 2 and 3 (pp. 32-53) of study report. Data not analyzed statistically.
2 Numbers reflect number of animals affected (days observed). Severity of signs was not indicated in study report.

The study authors reported that urine stains in both sexes, and emaciation, yellow cutis, body pallor and labored respiration in females, were observed with greater frequency in treated animals compared to controls, but that their relationship to treatment was unclear because of the lack of a dose-response. Most of these signs were first observed after the second year of treatment. TB-I does not consider the signs to be clearly treatment related due to the lack of dose-response. In females, the apparent increase in the incidence or earlier onset of the above signs in treated animals may be secondary to the increased incidence of large granular lymphocyte (LGL) leukemia observed in all treated groups, reflecting generally poorer health.

Survival at selected times during the study is shown below in Table 3:

TABLE 3: SURVIVAL [1]

	0 ppm	50 ppm	250 ppm	1000 ppm
MALES:				
Total animals in study	100	100	100	100
Scheduled sacrifice, total	75	71	71	70
(52-week)	10	10	10	10
(78-week)	9	9	10	9
(104-week)	56	52	51	51
Percent survival at termination[2]	70	65	64	64
Total deaths/moribund sacrifice	25	29	29	30
Mean survival time, days	699	695	701	699
FEMALES:				
Total animals in study	100	100	100	100
Scheduled sacrifice, total	81	65	71	76
(52-week)	10	10	10	10
(78-week)	9	8	9	10
(104-week)	62	47	52	56
Percent survival at termination[2]	78	59	65	70
Deaths/moribund sacrifice	19	34	29	24
Mean survival time, days	720	690	701	714

1 Data extracted from Table 1 (p. 31) of study report and from pathology report summary tables (Appendix 2).
2 Based on main sacrifice group, N = 80

No treatment-related effects on survival were observed. Survival was reduced in females at 50 ppm. The study authors reported that this decrease was statistically significant. [In acute oral toxicity studies, the LD_{50} has been shown to decrease (increased lethality) with decreasing concentration of glutaraldehyde within a certain concentration range (between 50% and 1% glutaraldehyde, % w/w in water). However, in this study TB-I agrees with the study authors that this was not a treatment-related effect because of the lack of other signs of toxicity at that dose other than reduced water intake and the low concentration (0.05%) of the dosing solution.]

2. Body weight

Animals were weighed prior to initiation of treatment and weekly thereafter, then immediately prior to sacrifice.

Results - Mean body weights at selected intervals and cumulative body weight gain for males and females are shown below in Table 4:

TABLE 4: SELECTED MEAN BODY WEIGHT VALUES AND CUMULATIVE GAIN (GRAMS)[1]

DOSE, PPM:	0	50	250	1000
Males:				
Initial wt.	155.7	156.4	155.3	155.5
Week 1	181.9	182.0	180.7	175.7**
Week 13	302.2	302.3	298.1	290.3**
Week 27	339.6	337.9	331.0**	323.6**
Week 53	382.5	380.1	369.7**	357.3**
Week 79	411.0	404.8	394.1**	374.6**
Week 103	387.0	372.7*	366.1**	351.3**
CUMULATIVE GAIN, 1 YR	227.0	223.7	214.7**	202.2**
2 YR	230.6	216.4*	213.1** (-7.6%)[2]	197.4** (-14.4%)
Females:				
Initial wt.	111.9	111.0	110.6	110.8
Week 1	125.3	123.8*	123.6**	122.3**
Week 13	181.7	180.8	179.8	175.6**
Week 27	192.5	191.3	191.5	186.5**
Week 53	214.2	215.6	215.3	206.3**
Week 79	247.9	247.1	251.0	235.1**
Week 103	265.3	264.4	261.7	244.8**
CUMULATIVE GAIN, 1 YR	102.4	104.8	104.8	95.5**
2 YR	153.5	154.5	151.8	133.1** (-13.2%)

1 Data taken from Tables 5 through 8 (pp. 55-71) of study report
2 Numbers in parentheses indicate % decrease at 2 yr compared to controls
* $P \leq 0.05$ ** $P \leq 0.01$

Results - In males at 250 ppm, mean body weight was slightly but statistically significantly lower than controls for most of the study beginning at week 6. Mean body weight was 5.4% less than controls at week 103. Body weight gain showed a slight but statistically significant decrease during the first year (also 5.4% less than controls) and a more pronounced decrease during the second year (-7.6%). At 1000 ppm, body weight and weight gain were decreased throughout the study (-9.3%, body weight and -14.4%, body weight gain at week 103). At 50 ppm, statistically significantly decreased mean body weight was observed only at week 103 and gain at 67 to 71 and 103 and TB-I agreed with the study authors that this was not treatment related. In females, no effects except sporadic decreases were observed at 250 ppm. At 1000 ppm, statistically significantly reduced body weight and weight gain were observed throughout the study (-7.8%, body weight and -13.3%, body weight gain at week 103). The decreases were due in part to reduced food consumption.

3. Food consumption

Food consumption for each animal was measured weekly during the first 13 weeks and every other week thereafter.

Results - Selected food consumption values during the study are shown below in Table 5:

TABLE 5: SELECTED FOOD CONSUMPTION VALUES (G/ANIMAL/DAY)[1]

	0 PPM	50 PPM	250 PPM	1000 PPM
MALES:				
Week 0-1	16.7	17.1*	16.6	15.2**
Week 12-13	15.6	15.7	15.3*	14.9*
Week 26-27	18.7	18.7	18.1*	17.6**
Week 52-53	19.4	19.8	19.5	18.8**
Week 78-79	19.2	17.9**	17.7**	18.0**
Week 102-103	17.1	16.6	16.4	16.7
FEMALES:				
Week 0-11	12.0	11.7**	11.6**	10.8**
Week 12-13	11.3	11.3	11.2	10.6**
Week 26-27	13.3	13.2	12.9**	12.6**
Week 52-53	13.9	13.7	13.6**	13.0**
Week 78-79	13.5	13.7	13.8	12.9**
Week 102-103	13.8	14.8	13.5	13.3

1 Data extracted from Tables 13 and 14 (pp. 93 - 102) of study report
* $p \leq 0.05$ ** $p \leq 0.01$

At 250 ppm, slight but statistically significant reductions (approximately -2% to -8%) in mean food consumption were observed in males (weeks 6, 8, 11 - 20, 26 - 30, 36, 38, 42, 44, 46, 48, 58, 60, 70, 76, 78 and 92) and females (weeks 1, 5, 6, 11, 14, 18, 20, 22, 24, 26, 36, 50, 52, 54, 62, 70, 72, 82, 94 and 96). At 1000 ppm, mild but significantly reduced food consumption (-2% to -12%) was sustained throughout most of the study for both males and females. Sporadic statistically significant variations (including several increases) in food consumption at 50 ppm were not considered treatment-related. Decreased food consumption may have been related in part to sensory and/or gastric irritation from repeated exposure to glutaraldehyde.

4. Water intake: Water intake for each animal was measured weekly during the first 13 weeks of the study and every other week thereafter.

Results.- Mean water intake from selected intervals during the study is shown below in Table 6:

TABLE 6: SELECTED WATER INTAKE VALUES (G/ANIMAL/DAY)[1]

	0 ppm	50 ppm	250 ppm	1000 ppm
MALES:				
Week 0-1	23.1	23.2	21.3**	17.3**
Week 12-13	22.3	22.2	19.9**	18.1**
Week 26-27	24.0	23.8	22.0**	19.3**
Week 52-53	24.2	24.7	23.4**	21.2**
Week 78-79	26.8	25.0**	24.1**	23.1**
Week 102-103	28.3	27.3	23.9**	21.9**
FEMALES:				
Week 0-1	18.8	18.5	16.7**	13.6**
Week 12-13	22.4	21.7	18.9**	14.6**
Week 26-27	23.4	22.5*	19.7**	16.8**
Week 52-53	23.1	22.3	20.3**	17.7**
Week 78-79	25.5	25.2	22.7**	19.1**
Week 102-103	24.4	24.2	21.8**	19.0**

1 Data extracted from Tables of study report (pp. 73-82)
* $p \leq 0.05$ ** $p \leq 0.01$

Water intake was statistically significantly decreased throughout the study at 250 and 1000 ppm in both sexes. The decrease was more pronounced in females at both doses: for males, decreases ranged from -6 to -16% at 250 ppm and from -9% to -25% at 1000 ppm compared to -6 to -22% (250 ppm) and -21 to -37% (1000 ppm) for females. At 50 ppm, statistically significant decreases ranging from -2 to -12% less than controls were observed sporadically in males. Decreases were more frequent in females at 50 ppm (-3% to -12% of controls in approximately 1/3 of interval measurements). TB-I agrees with the study authors that the decreases at 250 and 1000 ppm were due to unpalatability of glutaraldehyde, and possibly sensory irritation. The decreases at 50 ppm were less consistently observed, generally were not decreased by more than 8% and may indicate a threshold decrease due to unpalatability. The study authors determined that 50 ppm was the study LEL based on this effect.

5. Test compound intake: The study authors calculated mean weekly intake of glutaraldehyde based on water consumption and body weight. The average daily glutaraldehyde intake for the entire study was 3.6, 17.1 or 63.9 mg/kg/day (males) and 5.5, 25.1 or 85.9 mg/kg/day (females) at 50, 250 or 1000 ppm, respectively (see Tables 11 and 12 of study report).

6. Ophthalmoscopic examination

A complete indirect ophthalmologic examination was performed on all animals at pretest and on survivors prior to sacrifice.

Results - No treatment-related ocular effects were observed. Cataracts and corneal crystals were frequently observed among all dose groups.

7. Blood was collected under methoxyflurane anesthesia from the orbital sinus of 20 animals/sex/dose group (fasted for 16 hr) at Weeks 13, 26, 52, 78 and 104 for clinical analysis and hematology (no pretest samples were collected). The CHECKED (X) parameters were examined.

a. Hematology

X		X	
X	Hematocrit (HCT)*	X	Leukocyte differential count*, **
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)
X	Platelet count*		Reticulocyte count
Blood clotting measurements			Methemoglobin
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* Required for subchronic and chronic studies
** Control, high dose and moribund animals only

Results - Selected hematology parameters are shown below in Table 7:

TABLE 7: SELECTED HEMATOLOGY PARAMETERS[1]

SEX/PARAMETER		0 ppm	50 ppm	250 ppm	1000 ppm
MALES:					
Lymphocytes (cells/ml)	Week 13	7062	7067	7215	7325
	26	6024	6676*	6853**	6616*
	52	3544	3739	3588	3729
	78	2649	2756	2582	3035*
	104	2699	2278	2593	2963
Monocytes (cells/ml)	Week 13	538	508	489	530
	26	627	640	583	556
	52	552	392**	467	474
	78	438	484	430	483
	104	459	387	272	305
Lg. monocytes (cells/ml)	Week 104	1166	1917	5761*	6984*
FEMALES:					
Lymphocytes (cells/ml)	Week 13	4798	5741*	5043	5639*
	26	4324	4456	4815	4704
	52	2457	2362	2252	2546
	78	1752	1968	1789	2035
	104	1992	2384	2182	2612
Monocytes (cells/ml)	Week 13	295	472**	323	383
	26	470	472	497	421
	52	285	257	243	251
	78	324	362	253	337
	104	377	481	278	526
Lg. monocytes (cells/ml)	Week 104	1561	0	8905	1488

1 Data taken from Tables 15 - 24 (pp. 103 - 122) of study report
* $p \leq 0.05$ ** $p \leq 0.01$

TB-I agreed with the study authors that the sporadic statistically significant increases in lymphocytes and monocytes observed in the study were not related to treatment. Large monocytes showed significant increases at week 104 in males at 250 and 1000 ppm and were increased in females at 250 ppm. TB-I agreed with the study authors that this was probably related to the presence of LGL leukemia. There was considerable variability in large monocyte count among individual animals: the above means reflect counts greater than zero in 1, 3, 8 and 8 males and 3, 0, 4 and 4 females at 0, 50, 250 and 1000 ppm, respectively. The standard deviations for lymphocytes and monocytes were also relatively high at 104 weeks.

b. Clinical Chemistry

X		X	
	Electrolytes:		Other:
X	Calcium*	X	Albumin*
X	Chloride*	X	Blood creatinine*
	Magnesium	X	Blood urea nitrogen*
X	Phosphorus*		Cholesterol*
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
	Enzymes	X	Total/direct/indirect bilirubin
X	Alkaline phosphatase (ALK)	X	Total serum protein (TP)*
	Cholinesterase (ChE)		Triglycerides
X	Creatinine phosphokinase		Serum protein electrophores.
X	Lactic acid dehydrogenase (LDH)		
X	Serum alanine aminotransferase (also SGPT)*		
X	Serum aspartate aminotransferase (also SGOT)*		
X	Gamma glutamyl transferase (GGT)		
X	Glutamate dehydrogenase (GLDH)		
x	Sorbitol dehydrogenase (SDH)		

* Required for subchronic and chronic studies

Results - There were no biologically significant treatment-related effects on clinical chemistry parameters. There was a generally decreased tendency of several serum enzymes (SGOT, SGPT, ALK, GLDH) at 1000 ppm and occasionally at lower doses (data not shown). Statistically significant reductions in some enzymes (SGOT, SGPT, ALK) were observed in males at 1 - 3 time points (weeks 26, 52 and/or 78) at 1000 ppm. Statistically significant decreases in SGOT, SGPT or GLDH were observed in females at week 52 at 1000 ppm (also week 13, GLDH). The decreases observed at 1000 ppm ranged between -10% to -30% of controls except for GLDH in females, which was up to -50%. The study authors attributed the effects on serum enzymes to the reduced food consumption and body weight gain; however, decreases are not considered to be toxicologically significant. Sporadic statistically significant decreases in serum sodium, phosphorus, total protein and gamma globulin were observed but were not considered treatment-related due to the small magnitude of the change and sporadic occurrence.

8. Urinalysis

Urine was collected from fasted animals at Weeks 12, 25, 51, 77 and 103 (no pretest samples were collected). The CHECKED (X) urine parameters were examined.

X		X	
| X |	Appearance*	| X |	Glucose*
| X |	Volume*	| X |	Ketones*
| X |	Specific gravity*	| X |	Bilirubin*
| X |	pH	| X |	Blood*
| X |	Sediment (microscopic)*	| |	Reducing substances
| X |	Protein*	| X |	Urobilinogen

* Required for chronic studies

Results - Selected urinalysis parameters are shown below in Table 8:

TABLE 8: SELECTED URINALYSIS PARAMETERS[1]

SEX/PARAMETER		0 ppm	50 ppm	250 ppm	1000 ppm
MALES:					
Volume (ml)	Week 12	8.8	8.9	6.8**	5.9**
	25	8.4	9.6*	7.1**	6.4**
	51	11.1	10.4	8.4**	8.1**
	77	13.3	15.0	10.8	7.9**
	103	16.8	16.6	12.1	10.1**
Osmolality (mOsmo/kg)	Week 12	1947	1872	2335**	2669**
	25	2424	2309	2708**	2827**
	51	1949	1977	2290**	2473**
	77	1752	1501	1965	2416**
	103	1190	1257	1529*	1611*
Protein (mg)	Week 12 30	1	0	0	0
	100	7	10	5	0
	≥300	2	0	5	10
	Week 25 30	0	0	0	0
	100	7	6	7	5
	≥300	3	4	3	5
Bilirubin	Week 12 -	8	10	4	2
	+	2	0	6	8
	Week 25 -	6	6	6	2
	+	4	4	2	6
	++	0	0	2	2
FEMALES:					
Volume (ml)	Week 12	9.9	8.9	7.2**	5.8 **
	25	9.9	8.8	8.0*	5.3**
	51	11.3	13.6	9.1	7.2**
	77	12.6	13.9	11.1	8.3**
	103	11.2	14.6	13.4	9.4
Osmolality (mOsmo/kg)	Week 12	1495	1748	1985**	2598**
	25	1842	2017	2236**	2764**
	51	1690	1712	1984**	2483**
	77	1580	1570	1842	2226**
	103	1434	1345	1499	1795**
Protein (mg)	Week 12 30	3	0	0	0
	100	7	10	10	1
	≥300	0	0	0	9
	Week 25 30	2	2	0	0
	100	8	8	10	3
	≥300	0	0	0	7
Bilirubin	Week 12 -	10	9	6	0
	+	0	1	4	10
	Week 25 -	8	7	8	3
	+	2	3	2	7

1 Data taken from Tables 35- 44 (pp. 143 - 162) of study report

* $p \leq 0.05$ ** $p \leq 0.01$

Urine volume was significantly decreased and osmolality increased at 250 and 1000 ppm in both sexes. In males, decreases in urine volume ranged from -15% to -27% at 250 ppm and -24 to -41% at 1000 ppm. Osmolality was increased 12% to 28% at 250 ppm and 17% to 38% at 1000 ppm. Comparable magnitudes of change were observed in females. TB-I agreed with the study authors that this was probably due to reduced water intake rather than direct toxicity of glutaraldehyde. The study authors also attributed sporadic decreases in pH at 250 and 1000 ppm in males and 1000 ppm in females (data not shown) and increased urinary protein and bilirubin to the reduced urine output. However, because these effects were not sustained throughout the study, their relationship to decreased water consumption is unclear. Although decreased water consumption was observed at 50 ppm, there were no corresponding effects on urinalysis parameters.

9. Sacrifice and Pathology

All animals were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed.

X	X	X
Digestive system	Cardiovasc./Hemat.	Neurologic
| | Tongue	| X | Aorta*	| XX | Brain*+
| X | Salivary glands*	| XX | Heart*	| X | Periph. nerve*
| X | Esophagus*	| X | Bone marrow*	| X | Spinal cord (3 levels)*
| X | Stomach*	| X | Lymph nodes*	| X | Pituitary*
| X | Duodenum*	| X | Spleen	| X | Eyes (optic n.)*
| X | Jejunum*	| X | Thymus*	Glandular
| X | Ileum*	Urogenital	| XX | Adrenal gland*
| X | Cecum*	| XX | Kidneys*+	| X | Lacrimal gland
| X | Colon*	| X | Urinary bladder*	| X | Mammary gland*
| X | Rectum*	| XX | Testes*+	| X | Parathyroids*++
| XX | Liver *+	| X | Epididymides	| X | Thyroids*++
| X | Gall bladder*	| X | Prostate	Other
| X | Pancreas*	| X | Seminal vesicle	| X | Bone*
Respiratory	| X | Ovaries*+	| X | Skeletal muscle*
| X | Trachea*	| X | Uterus*	| X | Skin*
| X | Lung*	| X | Vagina	| X | All gross lesions
| X | Nose (nasal turbinates)		and masses*
| | Pharynx		
| | Larynx		

* Required for subchronic and chronic studies.

+ Organ weight required in subchronic and chronic studies.

Results -

a. Organ weight - Selected absolute and relative organ weights are shown below in Table 9:

TABLE 9: SELECTED ABSOLUTE (G) AND RELATIVE (%) MEAN ORGAN WEIGHT DATA[1]

		0 ppm	50 ppm	250 ppm	1000 ppm
Males:					
Kidney/wk 52	abs	2.680	2.680	2.739	2.802
	rel	0.783	0.754	0.795*	0.820**
wk 78	abs	2.809	2.972	2.781	2.867
	rel	0.712	0.767**	0.728	0.796**
wk 104	abs	2.994	2.791*	2.789	2.770**
	rel	0.851	0.809	0.825	0.860
Brain wk 104	abs	1.968	1.966	1.983	1.976
	rel	0.558	0.570	0.588*	0.612**
Heart wk 104	abs	1.252	1.195*	1.218	1.172**
	rel	0.354	0.345	0.361	0.363
Females:					
Kidney/wk 52	abs	1.708	1.675	1.672	1.732
	rel	0.843	0.838	0.858	0.908**
wk 78	abs	1.756	1.721	1.853	1.876*
	rel	0.767	0.746	0.762	0.862**
wk 104	abs	1.890	1.895	1.969**	2.026**
	rel	0.774	0.778	0.804	0.890**
Brain wk 104	abs	1.804	1.781**	1.801	1.773**
	rel	0.740	0.730	0.737	0.780**
Heart wk 104	abs	0.923	0.909	0.927	0.888*
	rel	0.377	0.373	0.380	0.391

1 Data taken from Tables 45 - 62 (pp. 163 - 180) of study report.
2 Numbers in parentheses indicate percent of controls; only values ≥10% are indicated
* $p \leq 0.05$ ** $p \leq 0.01$

In males at 1000 ppm, slightly increased relative kidney weights were observed at weeks 52 and 78 (5% and 12% above controls, respectively). A slight but statistically significant decrease (-6%) in mean absolute kidney weight was observed at week 104 at 1000 ppm, possibly reflecting decreased body weight. In females at 1000 ppm, increases in both absolute and relative kidney weight were observed, primarily at weeks 78 and 104 (absolute weights 7% above controls; relative weights 12 to 15% above controls). Sporadic statistically significant increases at 250 ppm were of small magnitude in males (1.4%, week 52, relative kidney weight) and females (4%, week 104, absolute kidney weight). The study authors attributed the increased kidney weights at 1000 ppm to be physiological adaptations to the chronically reduced water consumption and noted that similar increases were observed in the 90-day range-finding study.

Statistically significant effects on brain and heart weight in both sexes were not considered to be treatment related due to lack of corresponding microscopic pathology. The increased relative brain weights were primarily due to reduced body weight.

b. Gross pathology - The incidence of selected grossly visible lesions are shown below in Table 10:

TABLE 10: SELECTED GROSS LESIONS AT WEEK 104 (TERMINAL SACRIFICE ONLY)[1]

Sex:Observation	0 ppm	50 ppm	250 ppm	1000 ppm
MALES: N = ( )	(56)	(52)	(51)	(51)
Stomach				
color change, focal/multifocal	1	3	4	3
ulcerated	1	3	0	6
hemorrhage	2	0	0	2
FEMALES: N = ( )	(62)	(47)	(52)	(56)
Stomach				
color change, focal/multifocal	2	0	5	5
ulcerated	1	1	0	5
hemorrhage	1	0	0	4

1 Data taken from Tables 67 and 68 (pp. 188 to 196) of study report.

A slight increase in the incidence of gastric ulceration was observed in both males and females at 1000 ppm at terminal sacrifice (but not in interim sacrifice animals). Slight increases in the incidence of gastric color change were also observed in females at 250 and 1000 ppm and also in the interim sacrifice groups at these dose levels (data not shown); these correlated with some, but not all, incidences of ulceration or glandular hyperplasia. TB-I agreed with the study authors that the increased ulceration at 1000 ppm was probably a treatment-related gastric irritation effect, which is supported by the microscopic pathology data (see below). Glutaraldehyde is known to be irritating to the gastrointestinal lining.

c. Microscopic pathology - Selected nonneoplastic microscopic lesions in all animals of the main study group are shown in Table 11 below (for terminal sacrifice animals, only control and high dose routinely were assessed; other dose levels only when needed. Values for scheduled and unscheduled deaths are given separately since a combined statistical analysis was not performed):

TABLE 11: SELECTED NON-NEOPLASTIC MICROSCOPIC LESIONS IN ALL RATS OF MAIN (TERMINAL SACRIFICE) GROUP[1]

Sex:Lesion	0 ppm	50 ppm	250 ppm	1000 ppm
MALES:				
Stomach N = ( )[2]	(56/25)	(9/29)	(12/28)	(51/30)
mucosal hyperplasia	1/1[2]	1/3	1/2	7*/3
keratin cyst	0/0	0/0	0/0	4*/0
lymphoid infiltrates	1/1	0/2	1/0	6/0
ulcer	1/3	1/1	0/2	1/2
Liver N = ( )	(56/25)	(52/29)	(50/29)	(51/30)
peliosis	0/0	0/1	3/0	4*/0
fatty change	18/13	14/6	13/5	18/7
Kidney N = ( )	(56/25)	(52/29)	(51/29)	(51/30)
tubular pigmentation	4/2	6/4	4/2	15**/10
tubular basophilia	53/12	42*/4	43/8	45/9
interstitial nephritis	51/17	49/10	44/14	41/13
Bone marrow N = ( )	(56/25)	(1/34)	(0/29)	(51/30)
hyperplasia	7/13	0/10	0/9	16*/18
FEMALES:				
Stomach N = ( )	(62/18)	(1/34)	(6/29)	(56/24)
mucosal hyperplasia	1/0	0/2	1/0	7*/7
keratin cyst	0/0	0/0	0/0	0/0
lymphoid infiltrates	1/0	0/0	0/0	2/0
ulcer	0/1	1/6	0/7	3/14
Liver N = ( )	(62/19)	(47/35)	(52/29)	(56/24)
peliosis	0/0	2/0	0/0	1/0
fatty change	10/5	13/16	19*/12	6/12
Kidney N = ( )	(62/19)	(47/35)	(52/29)	(56/24)
tubular pigmentation	12/6	11/11	29**/11	41**/16
tubular basophilia	17/2	19/6	28**/3	38**/5
interstitial nephritis	35/3	27/4	34/3	43*/4

1 Data taken from Tables 75 and 76 (pp. 237 - 260) of study report.
2 No. surviving animals/no. unscheduled death animals examined. Statistical analyses of combined surviving animals/deaths was not performed.
* $p \leq 0.05$ ** $p \leq 0.01$

No treatment-related findings were observed at 52 or 78 weeks in the interim sacrifice animals. At 104 weeks, small but statistically significant increases in gastric mucosal hyperplasia and increases in other lesions consistent with irritation to the gastric mucosa were observed in both males and females at 1000 ppm. Mucosal hyperplasia (males and females) and ulcer (females) were also increased in the unscheduled death animals. Although the incidence of these lesions was low, TB-I agrees with the study authors that they are related to treatment because of the known irritant properties of glutaraldehyde. In the kidney, statistically significant increases in tubular pigmentation were observed at 250 and 1000 ppm in surviving females and 1000 ppm in surviving males (also increased among unschedule deaths). In addition, in surviving females renal tubular basophilia was increased at 250 and 1000 ppm and interstitial nephritis at 1000 ppm and bone marrow hyperplasia increased in surviving males at 1000 ppm (these lesions were not increased among unscheduled deaths). TB-I agreed with the study authors that these microscopic

effects were secondary to LGL leukemia since they are commonly associated with that disease. Although statistically significant increases in hepatic peliosis (males) and fatty change (females) were observed they are not considered treatment-related since peliosis is a common lesion in old male rats and fatty change in females did not show a dose-response.

Neoplastic - Selected neoplastic lesions are shown below in Table 12:

TABLE 12: INCIDENCE OF SELECTED NEOPLASMS IN ANIMALS OF MAIN GROUP[1]

Sex:Lesion:Severity	0 ppm	50 ppm	250 ppm	1000 ppm
MALES: (N = 100)				
Large granular lymphocyte leukemia, total[2]	43	51	40	46
Mild	6	15	2	5
Moderate	17	12	8	11
Marked	8	8	12	12
Severe	12	16	18	18
Mesothelioma, one/multiple sites (N = 100)	1	1	3	6
FEMALES: (N = 100)				
Large granular lymphocyte leukemia, total	24	41*	41*	53**
Mild	2	6	5	5
Moderate	8	11	10	17
Marked	7	5	6	15
Severe	7	19	20	16
Mesothelioma, multiple sites (N = 100)	0	0	1	0
Uterus (N =)	(100)	(53)	(45)	(99)
Adenocarcinoma	0	0	2	2

1 Data taken from Tables 83 and 84 (pp. 311, 312) of study report.
2 Statistical analyses and grading of severity of LGL leukemia based on data from spleen.
3 Identified in one or more locations (testes, epididymes, mesentery, spleen, etc.). Data not analyzed statistically
* $p \leq 0.05$ ** $p \leq 0.01$

In males, a high incidence of LGL leukemia was observed in all groups. Among affected males, a slightly higher incidence of LGL leukemia characterized as severe was observed at 250 and 1000 ppm (28%, 31%, 45% and 39% of tumor-bearing animals, control to high dose). In females, incidence was statistically significantly increased in all treated groups compared to controls. Severity of the neoplasia was increased only at 50 and 250 ppm but not 1000 ppm in females; the percent with LGL characterized as severe was 29%, 46%, 49% and 30% of affected females (control to high dose). A time-to-tumor analysis performed using analysis methods of the National Cancer Institute indicated that treatment with glutaraldehyde did not decrease onset of tumor incidence. A significant trend was observed in females when all females were analyzed, but not when normalized for the higher incidence of leukemia in females at terminal sacrifice. No significant differences were identified in males in any analysis. LGL was not observed at 52 weeks in either sex. Incidence of LGL at 72 weeks was 1, 3, 0 and 0 in males and 0, 1, 0 and 0 in females (control to high dose). The study report notes that a recent study from their laboratory reported an

incidence of 44% in female rats. Another study noted in the report had incidences up to 52% in female controls (personal communication, Haseman, 1991 and a published study by Hermansky et al., 1992). One text on Fischer 344 rat pathology[1] reports a range of 6 to 31% (mean 20.2%) for control females surviving to two years. It is noted that Fischer 344 male rats generally have a higher incidence of this neoplasm than females. This laboratory reported an incidence of 66% in males in a recent study (females, 44%). A range of incidence of 10 to 72% (mean 33.6%) was reported for males in cancer bioassay studies conducted at NTP between 1977 and 1987[1].

Inspection of individual animal data showed that mesothelioma was reported in one or more organs in 1%, 1%, 4% and 6% of all males on study (control to high dose). This tumor was found in the testes (1/100, 0/90, 3/88 and 4/100) and/or epididymes (1/100, 1/67, 2/88 and 4/100), mesentery (3/19 at 1000 ppm) as well as other organs (spleen, adipose tissue, pancreas, etc.). Mesothelioma of the testes/epididymes was observed in one control male at the 72 week sacrifice. However, TB-I notes that since not all organs were examined in all animals and some mesotheliomas were apparently not initially identified as gross lesions, the possibility exists that some mesotheliomas may have gone unreported. The study authors did not provide historical control data for mesothelioma, but the summary of NTP studies reported an incidence ranging from 1 - 10% in male Fischer 344 rats[1]. Mesothelioma was observed in only one female in the 250 ppm group that died on study due to this tumor.

Adenocarcinoma of the uterus was noted at an incidence of 0%, 0%, 4.4% and 2% (control to high dose; only about half of low and mid dose animals examined). The increase at mid and high dose was not statistically significant. The study authors also did not provide historical control data from their laboratory on the incidence of uterine adenocarcinoma, but the NTP laboratory summary reported a range of 0 - 4% in female F344 rats[1].

E. DISCUSSION: The study authors did not assign a NOEL to this study due to the reduction in drinking water observed at 50 ppm in both sexes. However, in the opinion of TB-I decreased water consumption at 50 ppm was most likely due to unpalatability of glutaraldehyde and is therefore noted, but not considered to be a treatment-related systemic toxicity effect. This is supported by the lack of other effects at that dose.

Dosing is considered adequate in this study based on body weight and food consumption effects and indications of gastric irritation in some animals. Microscopic lesions of the kidney were probably secondary to the large granular lymphocyte leukemia. Effects on urine volume and osmolality and kidney weight were probably secondary to the decrease in water consumption.

The increased incidence of LGL leukemia in all treated females was statistically significant according to the study author's analysis. No other statistically significant

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[1]Boorman, G.A., et al., eds. Pathology of the Fischer Rat, Reference and Atlas (1990) Academic Press.

increases in tumor frequency were observed. Apparent slight increases in mesothelioma (males) and in uterine adenocarcinomas, both within reported historical control range for the Fischer 344 rat, were also observed. The study authors did not consider the mesothelioma or uterine lesions treatment-related and considered the increased incidence of LGL leukemia in female rats to be of unclear toxicological significance due to the variable, sometimes high, incidence seen in controls and the unclear etiology of the disease. The HED RfD/Peer Review Committee will determine whether the tumor incidences warrant evaluation by the Cancer Peer Review Committee.

STUDY DEFICIENCIES: For some organs showing possible treatment-related effects (eg stomach) low and mid dose organs were not examined in all animals. No other significant study deficiencies were noted.

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